levels of phosphorus, potassium, calcium, and magnesium furnished the host plant.

Results also signify that the best growth response to effective inoculation and increased mineral nourishment was obtained with a treatment of 200 p.p.m. of inorganic nitrogen. With this nitrogen plants grew more rapidly and had larger leaves than did those grown without it. Presumably, the denser foliage and, hence, increased photosynthetic capacity of the plants were beneficial to symbiotic nitrogen fixation. No improvement in plant growth or total nitrogen content of the plants resulted from increasing the level of inorganic nitrogen from 200 to 400 p.p.m. It appears that high levels of inorganic nitrogen suppress symbiotic nitrogen fixation.

Apparently, the bean plant requires rather large amounts of divalent cations for maximum growth and best response to inoculation with rhizobia. Further study to determine a proper balance between potassium and the divalent cations, calcium and magnesium, appears warranted.

A point of practical importance to bean growers is the beneficial effect of high levels of calcium, magnesium, and phosphorus on pod yields. Increasing the level of phosphorus from 10 to 100 p.p.m. caused a 41% increase in pod yield from effectively inoculated plants, although the total nitrogen content of the plants was not materially affected (Table II). The responses in pod yields resulting from high levels of calcium and magnesium were even more spectacular. Plants furnished 300 p.p.m. of calcium and 100 p.p.m. of magnesium produced almost three times the yield of green pods harvested from those which received only 100 p.p.m. of calcium and 25 p.p.m. of magnesium.

In general, the results reported here agree with those obtained with other leguminous plants. Roberts and Olsen (*14*) showed that inoculated red clover plants fixed little nitrogen in the absence of ample potassium. Peanut plants grown on fertile soils fixed more nitrogen than did those grown on poor soils (*13*). Lynch and Sears (*11*) noted improved efficiency of rhizobia of *Lotus corniculatus* as a result of high fertility treatments given the host plant. In experiments of a similar nature, Ash (*3*) showed that alfalfa grown in the presence of 100 and 200 p.p.m. of phosphorus and potassium, respectively, fixed the largest amounts of nitrogen. On the other hand, the nitrogen-fixing ability of an ineffective strain was improved little by high fertility treatments.

All of the afore-mentioned results seemingly point to the conclusion that both effective rhizobia and adequate plant food are essential for best nitrogen fixation. Whereas nitrogen-fixing abilities of mediocre strains may be improved by high fertility treatments, fertilization is not a good substitute for effective rhizobia, or vice versa.

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INSECTICIDE ANALYSIS

The Colorimetric Determination and Paper Chromatography of Some Aromatic Carbamates

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THE FORMATION of naphthol blue from 1-naphthol and *p*-nitrobenzenediazonium fluoborate has been used as the basis of a colorimetric method of analysis for Sevin (1-naphthyl *N*-methylcarbamate) (*3*). Since there are many other compounds which will react with a suitable diazonium salt to produce a colored product, this study was undertaken to establish a method of analysis for similar carbamate or other pesticidal compounds having a potentially reactive moiety. Four cyclic carbamates (Geigy Chemicals Co.) which have such a potentially reactive moiety were obtained. Upon hydrolysis, Isolan [dimethyl 5-(1-isopropyl-3-methyl pyrazolyl) carbamate], Dimetilan [dimethyl 5-(3-methyl pyrazolyl) carbamate], and Pyrolan [dimethyl 5-(1-phenyl-3-methyl pyrazolyl) carbamate] yield pyrazols. The fourth insecticidal carbamate, Dimetan (dimethyl 5,5-dimethyl-3-oxo-1-cyclohexen-1-yl carbamate) hydrolyzes to the 5,5-dimethyldihydroresorcinol. Another insecticidal carbamate, H-5727 (3-isopropyl phenyl *N*-methylcarbamate) (Hercules Chemical Co.), yields a reactive phenol. The moieties released by hydrolysis are coupled with *p*-nitrobenzenediazonium fluoborate to give colored products suitable for colorimetric estimation.

Although the diazo coupling reaction is not a general one (*4*), several examples of phenols, which will react under the conditions outlined to give a suitable colored compound, are given in Table I. Carbamates or other pesticides containing these phenols may thus be determined by this method. In general, it is first necessary to unmask the phenol by

A colorimetric method of analysis for the insecticides Isolan, Pyrolan, Dimetilan, Dimetan, H-5727, and several common phenols is described. Alkaline hydrolysis of the carbamate insecticides produces aromatic moieties which are reacted with *p*-nitrobenzenediazonium fluoborate to produce colored compounds suitable for colorimetric estimation. Phenols are reacted directly in acid solution with *p*-nitrobenzenediazonium fluoborate and are estimated colorimetrically as the more intensely colored basic solutions. A paper chromatographic system for the separation of the insecticidal carbamates is described. The insecticidal carbamates are detected on paper by reaction with the fluoborate.

suitable hydrolysis in alkali to give the reactive free phenol. Combinations of the free phenol and the esterified phenol, such as may be encountered in metabolism studies, may be analyzed separately by coupling the phenols in acid to estimate the free phenol content, followed by hydrolysis and coupling to estimate the total phenol content.

Procedure

Isolan and Pyrolan. A stock solution of carbamate is prepared to contain 100 γ per ml. in methanol. An aliquot is pipetted into a 10-ml. volumetric flask, to which is added 1.0 ml. of 0.5N aqueous sodium hydroxide. Hydrolysis is carried out by heating in a steam bath for 20 minutes. After cooling, 1.0 ml. of 1N aqueous sodium hydroxide is added, followed by 1.0 ml. of 0.1% (w./v.) methanolic *p*-nitrobenzenediazonium fluoborate solution, and the resulting solution is diluted to 10.0 ml. with absolute methanol. The absorbance of the solution is measured in the spectrophotometer at the respective wave lengths (Table II).

Dimetilan. A stock solution in carbamate is prepared to contain 100 γ per ml. in ethyl alcohol instead of methanol. The same procedure as for Isolan and Pyrolan is carried out.

Dimetan. An aliquot of a methanolic stock solution containing 100 γ per ml. is pipetted into a 10-ml. volumetric flask to which are added 1.0 ml. of 10N aqueous sodium hydroxide and 1.0 ml. of ethylene glycol. The resulting solution is mixed and heated on a hot plate for 1 hour at approximately 100° C. After being cooled to room temperature, 4.0 ml. of glacial acetic acid is added. It is cooled again and 1.0 ml. of 0.1% (w./v.) methanolic *p*-nitrobenzenediazonium fluoborate solution is added. After 15 minutes, the solution is diluted to 10.0 ml. with absolute methanol and measured in a spectrophotometer at 390 mμ.

H-5727. An aliquot of a methanolic stock solution of carbamate containing 100 γ per ml. is pipetted into a 10-ml. volumetric flask, to which is added 0.5 ml. of aqueous 0.5N sodium hydroxide solution. After standing at room temperature for 10 minutes, 1.0 ml. of a 0.1% (w./v.) methanolic *p*-nitrobenzenediazonium fluoborate solution is added, and the resulting solution is diluted to 10.0 ml. After 15 minutes, the colored solution is measured in a spectrophotometer at 500 mμ.

Phenols. An aliquot of a methanolic stock solution of the phenol containing 100 γ per ml. is pipetted into a 10.0-ml. volumetric flask, to which is added 1.0 ml. of 0.5N aqueous acetic acid, followed by the addition of 1.0 ml. of a 0.1% (w./v.) methanolic *p*-nitrobenzenenediazonium fluoborate solution. The solution is mixed and allowed to stand for 15 minutes at room temperature. The final colored solution is produced by the addition of 1.0 ml. of 2N aqueous sodium hydroxide solution and dilution to 10.0 ml. with methanol. The final solution is measured at the wave length noted in Table I for the respective phenol.

In general, the precautions previously noted in the analysis of Sevin by *p*-nitrobenzenediazonium coupling (*3*) must also be observed in this analysis. The stability of the diazonium salt reagent is especially critical and therefore it is recommended that a fresh solution be prepared just prior to analysis and that the reagent should be stored at 0° to 10° C. Analytical grade, absolute ethyl alcohol contains trace amounts of unknown materials which give a slight discoloration in the final colored product. It is thus recommended that analytical reagent grade, absolute methanol be utilized whenever possible. Many other phenols were examined during this investigation, but are not reported because the final colored product either decomposed too rapidly for convenient analysis or absorbed light in the same region of the spectrum as the diazonium salt reagent.

Standard curves obtained by several analysts showed uniform and similar results, and indicated that this method can be used to determine as little as 10 γ of the carbamate insecticides cited.

Separation and Detection of Carbamate Insecticides by Paper Chromatography

A normal diphase paper chromatographic system is utilized (*1*, *2*). One or 2 μl. of an acetone solution of the carbamate, containing 10 to 20 γ, are spotted on a strip of Whatman No. 4 chromatographic filter paper 1 inch wide and 10 inches long. The paper strip is coated with the stationary phase, glutaronitrile, in a 10% (v./v.) acetone solution. After the acetone has evaporated at room temperature, the chromatogram is developed by descending elution with diisopropyl ether saturated at room temperature with glutaronitrile.

Table I. Detectable Phenols

Compound	Maximum Absorbance, Mμ
Naphthols	
1-Naphthol	590
2-Naphthol	530
1,6-Naphthalenediol	620
Phenols	
Phenol	460
Thymol	540
Resorcinol	585
2-Nitroresorcinol	555
3-Isopropylphenol	500

Table II. Standard Curve for Carbamate Insecticides

Compound and Maximum Absorbance	Reagent Blank	Quantity, γ 10	20	30	40	50	60	70	80
Isolan (485 mμ)	100[a]	76.0	60.0	45.0	34.5	27.5	21.0	16.0	13.5
Pyrolan (475 mμ)	100[a]	74.0	55.5	42.0	31.0	23.0	17.0	12.5	——
Dimetilan (485 mμ)	100[a]	80.0	66.0	53.0	42.5	34.5	28.0	22.0	18.0
Dimetan (390 mμ)	89.2[b]	66.0	46.0	33.2	23.5	16.8	——	——	——
H-5727 (500 mμ)	92.0[b]	59.0	36.0	24.0	15.5	10.0	——	——	——

[a] % transmission recorded against the reagent blank as 100.
[b] % transmission recorded against a methanol blank as 100.

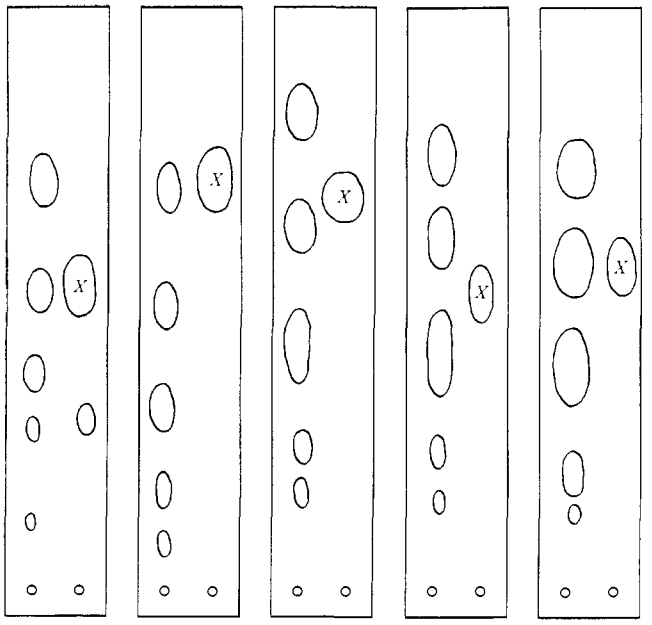

Figure 1. Mobilities of carbamate insecticides as observed in the chromatographic system

Approximately 0.5 hour is required for the solvent front to reach 15 cm. from the origin. The solvent is allowed to evaporate from the chromatogram and the dry chromatogram is sprayed with a 15% (w./v.) aqueous potassium hydroxide solution. The sprayed strip is then held over steam for 1 minute, followed by spraying with 1*N* acetic acid in methanol. After drying under an infrared lamp, a 0.1% (w./v.) methanolic *p*-nitrobenzenediazonium fluoborate solution is sprayed on the strip. The orange to red spot is intensified further by spraying with 15% (w./v.) aqueous sodium hydroxide solution. Gordon (*1*) has shown that a system of marker dyes gives convenient and reproducible reference positions. Thus a mixture of dyes, as described by Gordon (*1*), is also pipetted on the chromatogram prior to development. Spot movements are defined in relation to the standard dyes run simultaneously on the same strip. The dyes used in this chromatographic system are obtained from the coupling of diazotized 4-amino-2,5-diethoxybenzanilide plus 2-naphthol; diazotized 4-amino-2,5- diethoxybenzanilide plus 2-anilinoethanol; diazotized 4-benzoylamino - 2,5 - dimethoxyaniline, (Fast Blue RR), plus 2-anilinoethanol; and tetrazotized *o*-dianesidine (Naphthanil Diazo Blue B), plus 2-anilinoethanol. These dyes were used without further purification.

Figure 1 indicates the mobilities of the carbamate insecticides observed in the chromatographic system. Ten micrograms of each of the carbamates are easily detected.

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INSECTICIDE DETERMINATION

Diazotized Sulfanilic Acid Reagent for Endrin Analysis

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The colorimetrically undesirable side reaction between excess sulfanilic acid and diazotized sulfanilic acid can be prevented by addition of an excess of sodium nitrite, which is then destroyed by addition of ammonium sulfamate.

IN THEIR method for "Determination of Endrin in Agricultural Products and Animal Tissues," Bann and coworkers (*1*) employed a coupling reagent, prepared by mixing equal volumes of 0.5% sulfanilic acid in 50% acetic acid and 0.05% sodium nitrite solution. The reagent prepared in this manner contains a quantity of sulfanilic acid equal to 3.6 times the chemical equivalent of the sodium nitrite used. In our laboratory, diazotized sulfanilic acid was found to react with excess sulfanilic acid present, resulting in a strong background color. The intensity of the background color varied with the age of reagents and temperature.

Laboratory studies showed that an excess of sodium nitrite would diazotize sulfanilic acid completely, thus preventing the color-forming reaction. Further tests showed that if a slight excess of sodium nitrite was used in the diazotization of sulfanilic acid and the excess destroyed with ammonium sulfamate, there was little or no background color from the color-forming reagents.

Diazotized sulfanilic acid reagent is prepared as follows:

0.25% sulfanilic acid. Dissolve 0.25 gram of sulfanilic acid in 100 ml. of 60% glacial acetic acid in distilled water. Warm to 50° C. to aid solution of the acid.

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